



*Bulgarian Society  
of Physiological Sciences  
Sofia branch*

# *SCIENTIFIC MEETING*

## ABSTRACTS

*Sofia, 23 November, 2018*



**BULGARIAN SOCIETY  
OF PHYSIOLOGICAL SCIENCES  
*SOFIA BRANCH***

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**SCIENTIFIC MEETING  
ABSTRACTS**

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**23 November, 2018, Sofia, Bulgaria**



## **LOCAL ORGANIZING COMMITTEE**

Prof. Elena Dzhambazova, PhD

Assist. Prof. Milena Mihailova, PhD

Assist. Prof. Rene Mileva, PhD

Assist. Prof. Polina Mateeva, PhD

Assist. Prof. Boris Kadinov, PhD

# Programme Overview

## Oral presentations

**14.00 – 14.15** Welcome to the meeting

**14.15 – 14.30** Liliya Vitanova

**Interactions between serotonin and ionotropic GABA receptors in frog and turtle retina: an immunofluorescent study**

**14.30 – 14.45** Petia Kuppenova, Elka Popova, Veselina Mihaylova

**GABA<sub>A</sub> receptor-mediated modulation of oscillatory potentials in the ON- and OFF-response of the frog electroretinogram**

**14.45 – 15.00** Borislav Angelov, Boris Kadinov, Aleksandar Shkondrov

**Assessment the effects of flavonoids Alcesefoliside and Mauritianin isolated from *Astragalus monspessulanus* on isolated cerebral vessels**

**15.00 – 15.15** Borislav Assenov, Petia Peneva, Daniela Pechlivanova, Stela Georgieva, Elena Dzhambazova, Petar Todorov

**Effects of seven newly synthesized hemorphan analogues on the nociception in mice**

**15.15 – 15.30** Peter Raychev

**Equations in the teaching of resting membrane potential - traditions and alternatives**

**15.30 – 15.45** Natasha Ivanova, Lidia Kortenska, Jana Tchekalarova

**Effects of Agomelatine and chronic constant light on home cage activity and sleep/wake disturbances**

**15.45 – 16.00** Tsveta Stoyanova, Zlatina Nenchevska, Natasha Ivanova, Lidia Kortenska, Rumiana Mitreva, Jana Tchekalarova

**Study the effect of endurance training on depressive-like behavior and lipid peroxidation levels in a model of melatonin deficiency**

## INTERACTIONS BETWEEN SEROTONIN AND IONOTROPIC GABA RECEPTORS IN FROG AND TURTLE RETINA: AN IMMUNOFLUORESCENT STUDY

**Liliya Vitanova**

*Department of Physiology, Medical University of Sofia, 1431 Sofia, Bulgaria*

**The aim** of the present study was to elucidate the putative interactions between the serotonergic and the GABAergic systems in the lower vertebrate retinas.

**Materials and methods:** Experiments were performed on 12-14  $\mu\text{m}$  frozen frog and turtle retinal sections, processed with more than 10 different antibodies directed against serotonin, GABA and their membrane receptors by means of the indirect immunocytochemical method.

**The results** showed that some of the GABAergic retinal neurons possess the serotonin receptors 5-HT<sub>2A</sub> and 5-HT<sub>3A</sub> on their cell membrane, and vice versa: certain serotonergic neurons possess the ionotropic GABA<sub>A</sub> and GABA<sub>C</sub> receptors. A simple neuronal chart is proposed to explain how the activation of serotonin receptors on a subgroup of GABAergic amacrine cells can decrease the GABA<sub>C</sub> receptor mediated inhibition of another subgroup of GABAergic amacrine cells on the retinal bipolar cells' activity. As the bipolar cells are the main generator of ERG, we succeed also to demonstrate electrophysiologically that the stimulation of ionotropic GABA receptors at the background of applied serotonin decreases the inhibition on the bipolar cells and increases the b- and d-ERG waves. It proves the validity of this scheme.

**Conclusion:** As the retina is a natural biological model of CNS, the relationships between both neurotransmitter systems described here may elucidate their participation in certain brain functions and to explain the possible mechanisms of action of some antidepressant drugs which effect is based on stimulation of 5-HT<sub>2A</sub> and 5-HT<sub>3A</sub> receptors.

**Keywords:** Retina, neurotransmitters' interactions, serotonin, GABA<sub>A</sub> and GABA<sub>C</sub> receptors, 5-HT<sub>2A</sub> and 5-HT<sub>3A</sub> receptors



## **GABA<sub>A</sub> RECEPTOR-MEDIATED MODULATION OF OSCILLATORY POTENTIALS IN THE ON- AND OFF-RESPONSE OF THE FROG ELECTRORETINOGRAM**

**Petia Kупenova, Elka Popova, Veselina Mihaylova**

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**Introduction:** The oscillatory potentials (OPs) of the electroretinogram (ERG) are high-frequency, small-amplitude components, superimposed on the ERG b-wave (ON-response) and d-wave (OFF-response). In the full-field ERG, they are used to evaluate the proximal retinal activity. In the clinical practice, light flashes are usually used to obtain ERG. Therefore, ON- and OFF- OPs cannot be separately evaluated. There is little data concerning specific properties and putative selective modulation of ON- and OFF-OPs by different neurotransmitters.

**The aim:** In the present work, we studied the effects of GABA<sub>A</sub> receptor-mediated modulation of ON- and OFF-OPs in the retina of frog (*Rana ridibunda*).

**Materials and methods:** For this purpose, we recorded ERG using long-lasting (3s) stimuli presented at 30s intervals in the dark in order to fully separate the ON- and OFF-responses. The OPs were extracted offline using digital filters at a bandwidth of 20-300 Hz. Seven OPs (O1 to O7) were evaluated. The effects of three concentrations (1 μM, 10 μM and 50 μM) of the selective GABA<sub>A</sub> receptor blocker bicuculline were followed.

**Results:** The ERG ON- and OFF-OPs were affected by the GABA<sub>A</sub> receptor blockade in a similar manner and to a similar extent at each of the concentrations tested. While the early OPs (O1-O3) increased their amplitude, the late OPs (O4-O7) decreased their amplitude and eventually disappeared. The effects were partially reversible. The peak latencies of both ON- and OFF-OPs were not significantly changed.

**Conclusion:** Our results show that there is no significant asymmetry in GABAergic modulation of ON- and OFF-OPs, mediated through GABA<sub>A</sub> receptors.

**Acknowledgement:** This study was supported by Grant 58/03.05.2018 from the Council for Medical Science, Medical University of Sofia.

## ASSESSMENT THE EFFECTS OF FLAVONOIDS ALCESEFOLIZIDE AND MAURITIANIN ISOLATED FROM ASTRAGALUS MONSPESSULANUS ON ISOLATED CEREBRAL VESSELS

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**Introduction:** Flavonoids are a major group of secondary metabolites, widespread in the plant world. Flavonoids have been proven in a large number of *Astragalus* sp., most commonly localized in the aerial parts of plants. So far, antioxidant and neuroprotective activity of flavonoids, saponins and saponin mixtures of various species of the genus *Astragalus* – *A. corniculatus*, *A. hamosus* has been established. There is further evidence, that a flavonoid fraction from *A. complanatus* exhibits a well-expressed hypotensive effect, most likely due to a significant reduction in peripheral resistance and a decrease in angiotensin II plasma levels. It has been shown that Calcicosine can produce an endothelial-independent vasodilatation due to its action as a non-competitive calcium channel antagonist. Formonone also induces vasodilation through endothelium/NO-dependent and endothelium-independent mechanisms in isolated rat aorta experiments.

**The aim:** We set to evaluate the influence of flavonoids Alcesefoliside and Mauritianin on contractility of brain vessels.

**Materials and methods:** The substances Alcesefoliside and Mauritianin were isolated with butanol extraction from the aerial part of *Astragalus monspessulanus* subsp. *monspessulanus*. Arterial segments (*a. basilaris*) with length of 1.8 - 2 mm were mounted and tested on a dual wire myograph (model 410A, JP Trading, Denmark).

**Results and conclusion:** The examined substances lead to change the vascular tone. The insignificant effects of the studied two flavonoids could not yet be explained and do not correspond to the available data on the effect of total flavonoids on the cardiovascular system. Also, the mechanism of the intrinsic action of the investigated substances, as well as the opposite effect of their combined effects on vascular segments of *a. basilaris* remains unclear.

## EFFECTS OF SEVEN NEWLY SYNTHETIZED HEMORPHIN ANALOGUES ON THE NOCICEPTION IN MICE

**Borislav Assenov<sup>1</sup>, Petia Peneva<sup>2</sup>, Daniela Pechlivanova<sup>3</sup>, Stela Georgieva<sup>4</sup>, Elena Dzhambazova<sup>1</sup>, Petar Todorov<sup>2</sup>**

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**Introduction:** Endogenous hemorphin-related peptides are released from sequentially hydrolysed hemoglobin. They belong to the family of "atypical" opioid peptides with affinity for opioid receptors and morphinomimetic properties.

**The aim:** In the present study, seven new analogues of VV-hemorphin-5, modified by the non-proteinogenic and/or natural amino acids in position 1 (Ile or Aib) and 7 (Lys/Orn/Dap/Dab) were synthesized to investigate their potential antinociceptive activities.

**Materials and methods:** The experiments were carried out on young adult male ICR mice. For the assessment of the visceral nociception we have used acetic-acid-induced writhing test. Each peptide was injected intracerebroventricularly at doses of 50, 25 and 12.5 µg, 5 minutes before the injection of the irritant acetic acid, and the nociceptive reactions (writhes) were counted for 30 minutes.

**Results and conclusion:** All analogues showed a short lasting antinociceptive effect during initial 5 minutes of observation. The analogue V<sub>2</sub>, with a substitution of Gln by diaminopropanoic acid (Dap), showed a powerful and prolonged antinociceptive effect in all three doses used, while the other peptide analogues exerted more variable effects on the visceral nociception depending on the dose or time after the intracerebral injection.

**Key words:** VV-hemorphin-5 analogues, visceral nociception, mice, writhing test

**Acknowledgement:** Financial support from BG05M20P001-2.009-0015 "Support for the development of capacity of doctoral students and young researcher in the field of engineering, natural and mathematical sciences" funded by the operational programme "Science and Education for Smart Growth" 2014–2020 the co-financed by the European Union through the European Social Fund is gratefully acknowledged.



## **EQUATIONS IN THE TEACHING OF RESTING MEMBRANE POTENTIAL - TRADITIONS AND ALTERNATIVES**

**Peter Raychev**

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Medicine, Sofia University "St. Kliment Ohridski", 1407 Sofia, Bulgaria*

In the system of medical education physiology is a discipline of key importance based on dynamically progressing and changing field of scientific knowledge. Constant emergence and accumulation of new facts and concepts makes teaching of physiology challenging task where success and effectiveness are highly dependent on tutors' abilities periodically to reevaluate and improve training practices, learning objectives and even to revise whole paradigms in organizing, structuring and prioritizing essential parts of the curriculum. Resting membrane potential (RMP) is a concept of critical importance in physiology of excitable structures. Equations quantitatively characterizing RMP are widely used in teaching of physiology as a tool bridging the gap between fundamental biophysical phenomena and complex physiological processes. Application of different equations in modern teaching approaches towards RMP are studied and critically evaluated. Study is based on the review and content analysis of more than 20 modern medical textbooks of physiology.

**Key words:** teaching physiology, resting membrane potential, medical education, content analysis, Goldman equation, chord conductance equation, Mullins-Noda equation

## EFFECTS OF AGOMELATINE AND CHRONIC CONSTANT LIGHT ON HOME CAGE ACTIVITY AND SLEEP/WAKE DISTURBANCES

Natasha Ivanova, Lidia Kortenska Jana Tchekalarova

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**Introduction:** Recent data from our lab revealed that the antidepressant agomelatine can correct symptoms of depression and anxiety caused by chronic constant light (CCL) exposure.

**Aim:** The present study aims to further investigate whether agomelatine is able to reverse the negative changes on home cage activity and sleep/wake cycle induced by CCL.

**Methods:** Adult male Wistar rats (250-300 g) were subjected to CCL for three weeks. agomelatine/vehicle, injected intraperitoneally (i.p.) at a dose of 40 mg/kg for 3 weeks (4:00 p.m), started after at least 3 weeks of adaptation to Light/Dark and Light/Light regime, respectively. Home cage activity was recorded for 24 hours, using 8-channel DVR recorder, connected to a computer with software and 6, 2-megapixel HD-TVI cameras. Rats were implanted with epidural electrodes for electrographic activity record 24 h using Acknowledge software ACK100W (BIOPAC Inc., USA). Scoring was performed on 8-second epochs as being sleep. The duration of time spent in the three states: awake, rapid eye movement (REM) and NREM (none-REM) was recorded. T-test and two-way ANOVA were performed (significant difference  $p < 0.05$ ).

**Results:** Chronic constant light did not affect the total duration of home cage activity but exerted changes in its circadian rhythmicity. The duration of home cage motor activity was increased during the light phase and decreased during the dark phase, which was restored to control level by agomelatine treatment. Rats, exposed to CCL showed reduced duration of wake and increased duration of rapid eye movements (REM) sleep. Agomelatine reversed the changes of sleep, induced by continuous illumination, by decreasing the duration of REM sleep and increasing the NREM activity sleep duration.

**Conclusion:** Current data provide evidence that agomelatine is able to correct disturbances of sleep/wake cycle and home cage in a preclinical model of melatonin deficiency with comorbid depression.

**Key words:** Agomelatine, chronic constant light, sleep, wake

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## **STUDY THE EFFECT OF ENDURANCE TRAINING ON DEPRESSIVE-LIKE BEHAVIOR AND LIPID PEROXIDATION LEVELS IN A MODEL OF MELATONIN DEFICIENCY**

**Tsveta Stoyanova, Zlatina Nenchevska, Natasha Ivanova, Lidia Kortenska, Rumiana Mitreva, Jana Tchekalarova**

*Institute of Neurobiology, Bulgarian Academy of Sciences, 1113 Sofia*

**Introduction:** The rhythmic secretion of melatonin from the pineal gland is involved in the circadian dynamics of a number of physiological functions. In the recent decades, studies focused on disturbed circadian biological rhythms /chronopathology/, in terms of potential negative consequences, represent significant interest for researchers to disclose mechanisms for a number of pathologies from cells to organism. Experimental and clinical studies support the suggestion that melatonin has a pivotal role as a synchronizers of biological circadian rhythms as well as its activity as a powerful endogenous antioxidant. The products of lipid peroxidation and endogenous antioxidants have a circadian rhythm of formation and activity, respectively. Endurance training has a direct impact on affective behavior and systemic markers of oxidative stress in different diseases. So far, there is no experimental data about the effect of aerobic exercise on depressive behavior and oxidative stress-related enzymes in a model of melatonin deficiency, including pinealectomy.

**The aim** of the present study was to investigate the impact of systemic physical exercises on diurnal rhythm of affective behavior and lipid peroxidation levels in a rat model of melatonin deficiency induced by pinealectomy.

**Materials and methods:** Pinealectomy was performed according to the method described by Hoffman and Reiter (1965) in male adult Wistar rats. The endurance training was conducted on a treadmill for a 6-weeks period. Depressive-like behavior was assessed by saccharine preference test and forced swimming test (FST) described in our previous studies. Lipid peroxidation levels were determined in plasma by ELISA.

**Results:** Pinealectomy lead to a depressive- like behavior. Pinealectomy caused a significant increase of plasma lipid peroxidation. The endurance training positively affected depressive behavior and mitigated the increased oxidative stress resulting from melatonin deficiency.

**Conclusion:** Taken together, our results revealed that the physical exercise can exert antidepressant and antioxidant effects in a rat model of of melatonin deficiency induced by pinealectomy.

**Key words:** lipid peroxidation, melatonin deficiency, depressive-like behavior, rats

**Acknowledgements:** This work was supported by the National Science Fund of Bulgaria (research grant # № DN 03/10; DM 11/4; DN 12/6).

## Author Index

Angelov, B., **6**  
Assenov, B., **7**  
Dzhambazova, E., **7**  
Georgieva, S., **7**  
Ivanova, N. **9, 10**  
Kadinov, B., **6**  
Kortenska, L., **9, 10**  
Kupenova, P., **5**  
Mihaylova, V., **5**  
Mitreva, R., **10**  
Nenchovska, Z., **10**  
Pechlivanova, D., **7**  
Peneva, P., **7**  
Popova, E., **5**  
Raychev, P., **8**  
Shkondrov, A., **6**  
Stoyanova, Ts., **10**  
Tchekalarova, J., **9, 10**  
Todorov, P., **7**  
Vitanova, L., **4**

